

# Physiological potential of popcorn seeds submitted to water stress after treated with bioregulator

## Potencial fisiológico de sementes de milho pipoca submetidas ao estresse hídrico após tratamento com biorregulador

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### Abstract

Seed performance is one of the key points for high productivity in crop development. The objective of this study was to evaluate the physiological potential of popcorn cultivar seeds (IAC 125 and BRS-Ângela) treated with Stimulate<sup>®</sup> bioregulator under water stress. Popcorn seeds were submitted to different osmotic potential levels 0; -0.1; -0.3; -0.6 and -0.9 MPa and induced by mannitol, after being treated with bioregulator at the dose of 1.5 L 100 kg<sup>-1</sup> of seeds. The experiment was conducted in a completely randomized design, in a 5 × 2 × 2 factorial design (osmotic potential levels x cultivars x absence and presence of bioregulator). The variables evaluated were: first count and final count of germination standard, shoot length, primary root length, and dry biomass of seedlings. Treating popcorn seeds with bioregulator is inefficient in improving the physiological potential under normal conditions and little water restriction, but under conditions in which water may be limited, the use of bioregulator positively influences germination, growth, and initial performance of seedlings.

**Key words:** Germination. Growth regulator. Water restriction. *Zea mays* L.

### Resumo

O desempenho de sementes é um dos pontos chave para que o desenvolvimento da cultura se transforme em alta produtividade. Este trabalho teve por objetivo avaliar o potencial fisiológico de sementes de cultivares de milho pipoca (IAC 125 e BRS-Ângela) tratadas com biorregulador Stimulate<sup>®</sup>, sob estresse hídrico. Sementes de milho pipoca foram submetidas a diferentes níveis de potencial osmótico 0; -0,1; -0,3; -0,6 e -0,9 MPa, induzidos pelo manitol, após serem tratadas com biorregulador na dose de 1,5 L 100 kg<sup>-1</sup> de sementes. O experimento foi conduzido no delineamento inteiramente casualizado, em esquema fatorial 5 × 2 × 2 (níveis de potencial osmótico x cultivares x ausência e presença do biorregulador). As variáveis avaliadas foram: primeira contagem e contagem final do teste padrão de germinação, comprimento da parte aérea, comprimento de raiz primária e biomassa seca das plântulas. O tratamento de sementes de milho pipoca com biorregulador é ineficiente na melhoria do potencial fisiológico em condições normais e de pouca restrição hídrica, porém em condições em que a água possa ser limitada, o uso do biorregulador influencia positivamente na germinação, crescimento e desempenho inicial das plântulas.

**Palavras-chave:** Germinação. Regulador de crescimento. Restrição hídrica. *Zea mays* L.

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## Introduction

Popcorn is appreciated in Brazil and classified as a delicacy. Its consumption varies from year to year, a fact that influences the extension of plantations, the prices paid to producers and, consequently, the product import or export (CAETANO, 2016). There are no official data on popcorn production, productivity, or planted area. These values are accounted for together with the common corn crop in the harvest and interim harvest (CONAB, 2018). However, recent advances in this type of cultivation point to Mato Grosso as the main national producer. In this state, climatic conditions such as rainfall during the vegetative phase and dry weather in the post-maturation phase provide high grain quality (CARVALHO, 2015).

In the rest of the country, popcorn is still considered a product of family farming and cultivated in small areas, except for some large corporate farmers who, due to their capacity to invest in irrigation, meet the demands of packaging companies that make the product available in the market (PEREIRA FILHO et al., 2016). However, the good prospects for expansion should be highlighted, since this culture is a differentiated market niche.

Seed performance is a crucial point for the development of any crop, with effects on productivity (MACHADO et al., 2001; SCHEEREN et al., 2010; ABATI et al., 2014). However, considering Brazilian soil and climatic conditions, practically all seeds are subject to changes in metabolism when exposed to adverse field conditions (TAIZ; ZEIGER, 2009). According to Carvalho and Nakagawa (2012), intrinsic factors such as viability, longevity, sanity, as well as environmental factors such as water, temperature, and oxygen can alter seed germination speed, percentage, and uniformity.

Water is one of the most influential factors for germination because its presence triggers a series of essential biological and physiological processes (KHAJEH-HOSSEINI et al., 2003). According to Kramer (1974), great plant changes occur in water deficit situations, and they may vary according to

its severity, duration, nature, the plant genotype, and development stage. The implications of water availability extend to seedling development and may restrict it due to changes in cellular turgidity (KAPPES et al., 2010).

In laboratory conditions, water stress during germination has been simulated with mannitol solutions in different species (COSTA et al., 2004; MACHADO NETO et al., 2004; CUSTÓDIO et al., 2009; COELHO et al., 2010; KAPPES et al., 2010; CHRISTOVAM et al., 2015), because it is a non-toxic, chemically inert sugar (MACHADO NETO et al., 2004).

Given the above, alternative technologies capable of reducing stress effects during crop early development stage and of maintaining a suitable seedling establishment in the field become relevant from a practical and economic point of view. Among the alternatives, the application of natural or synthetic bioregulators or growth regulators has shown efficiency in productivity increase, for altering vital and structural processes of plants or seeds (LACA-BUENDIA, 1989).

According to Vieira and Castro (2002), due to the composition, concentration, and proportion of the bioregulatory substances present, there may be stimuli to cell division and elongation and increased absorption of water and nutrients by the roots, promoting plant development.

In the literature, although several studies point out that bioregulators have no effect on seed germination, such as the results found by Vieira and Santos (2005) in cotton seeds, others have observed positive effects in their use. These authors verified that the bioregulator treatment did not influence seedling germination and dry mass. However, favorable results for the use of these compounds were found by Ferreira et al. (2007), with effects on corn seed vigor, and Moterle et al. (2011) in soybean seeds.

Barbieri et al. (2014) and Abati et al. (2014) highlighted that Stimulate<sup>®</sup> had no effect on germination when associating the bioregulator

effect in corn and wheat seeds under water stress situations, respectively. However, vigor is positively influenced by the application, and its effect depends on the stress level and the genotype used (BARBIERI et al., 2014). Yet, there is a lack of studies that emphasize the real implications of growth regulators in these situations.

Thus, the objective of this study was to evaluate the bioregulator effect on the physiological potential of popcorn seeds when submitted to water stress conditions.

## Material and Methods

The experiment was carried out in the Seed Testing Laboratory at the University Center of Ingá, located in Maringá, State of Paraná (Brazil). Two popcorn cultivars were tested: BRS-Ângela and IAC125, from the 2015/2016 harvest.

The cultivar seeds were divided into two distinct lots, one of which was treated with bioregulator at a dose of 1.5 L 100 kg<sup>-1</sup> of seeds, according to the manufacturer's recommendation; and the other remained untreated and was considered as control. The bioregulator used was Stimulate<sup>®</sup>, which consists of 0.005% indolebutyric acid (auxin), 0.009% kinetin (cytokinin), and 0.005% gibberellic acid (gibberellin) (STOLLER DO BRASIL, 1998).

Then, 'germitest papers' were moistened in a solution containing the osmotic potentials of 0 (distilled water only); -0.1; -0.3; -0.6; -0.9 MPa, induced by the application of mannitol at concentrations of 0.0; 7.450; 22.3513; 44.7026; 67.540 g L<sup>-1</sup> distilled water, respectively. Osmotic potential zero was used as a control. Saline solutions were prepared with deionized and distilled water, and mannitol concentrations were obtained from the Van't Hoff equation cited by Salisbury and Ross (1992).

After the treatments, seeds were submitted to tests to evaluate the physiological potential, which were conducted in a completely randomized design, in a factorial design 5 × 2 × 2 (osmotic potential level x cultivars x in the absence and presence of

bioregulator).

The tests used to evaluate the physiological potential were:

Germination test (%) - performed with four subsamples of 50 seeds each, with water volume 2.5 times that of dry paper mass. The prepared rolls were placed in a Mangelsdorf type germinator, at a constant temperature of 25 ± 2°C. The percentage of normal seedlings was analyzed on the seventh day of the experiment, according to the Rules for Seed Testing (BRASIL, 2009). The first germination count was performed on the fourth day after the test assembly, adopting the same methodology used for the germination test (BRASIL, 2009).

Seedling length - in the evaluation of seedling length, five replicates of 20 seeds were used for each treatment, and then placed to germinate under the same germination test conditions. The seeds were distributed in the longitudinal direction of the leaves, with the micropyle facing the lower end of the substrate. The rolls prepared were placed in a germinator at 25 ± 2 °C. The seedling length (primary root and shoot) considered normal was evaluated on the seventh day, using a millimeter ruler; results were expressed in cm seedlings<sup>-1</sup> (NAKAGAWA, 1999).

Dry biomass - the average dry biomass (g seedling<sup>-1</sup>) of seedlings was obtained after length evaluation. For this test, the normal seedlings obtained in the length test were placed in paper bags, properly identified and oven-dried with forced air circulation adjusted at 80 ± 2 °C, for 24 h, and then weighed in an analytical balance (0.001 g) (NAKAGAWA, 1999).

The data were submitted to analysis of variance and the means were compared by the F test, at 5% probability for the cultivar and bioregulator factors. The behavior of variables as a function of osmotic potential levels for each cultivar was analyzed by polynomial regression. Analyses were performed through the software System for Analysis of Variance - SISVAR (FERREIRA, 2011).

## Results and Discussion

There was a significant interaction between bioregulator and osmotic potentials x cultivars when observing the germination percentage of normal

seedlings in the first and final count, shoot length, primary root length and dry biomass of seedlings (Table 1). The application of bioregulator affected the popcorn seeds physiological performance under both normal and stress conditions.

**Table 1.** Means of normal seedling percentage in the first and final counts of the germination test, seedling shoot and primary root length, and seedling dry biomass for two popcorn cultivars in response to five levels of osmotic potential in a mannitol solution, with and without bioregulator.

Cultivars	Bioregulator	Osmotic Potential (MPa)				
		0.0	-0.1	-0.3	-0.6	-0.9
First Germination Count (%)						
BRS-Ângela	Without	33B	27A	27A	8A	0A
	With	65A	27A	13B	4B	1A
IAC 125	Without	59B	48A	35A	14A	5A
	With	77A	49A	40A	11A	2A
Germination (%)						
BRS-Ângela	Without	89B	92A	86A	82B	75B
	With	99A	94A	91A	92A	88A
IAC 125	Without	88A	89A	82A	78B	64B
	With	92A	90A	85A	90A	78A
Shoot length (cm)						
BRS-Ângela	Without	16.13A	12.80A	11.36A	5.91B	4.06B
	With	12.75B	13.59A	9.94B	8.06A	6.46A
IAC 125	Without	17.70A	13.40A	11.32A	6.19B	4.10B
	With	15.44B	14.38A	9.12B	9.13A	9.38A
Primary root length (cm)						
BRS-Ângela	Without	21.33A	19.96A	19.58A	15.12B	9.79B
	With	19.20B	21.25A	18.55A	17.94A	15.62A
IAC 125	Without	20.32A	21.02A	18.74A	10.68B	7.75B
	With	19.81A	18.20B	17.44A	15.13A	13.86A
Dry biomass (g)						
BRS-Ângela	Without	0.049A	0.041A	0.038A	0.030B	0.025B
	With	0.044B	0.043A	0.039A	0.039A	0.033A
IAC 125	Without	0.043A	0.037A	0.036A	0.027B	0.022B
	With	0.042A	0.036B	0.035A	0.035A	0.035A

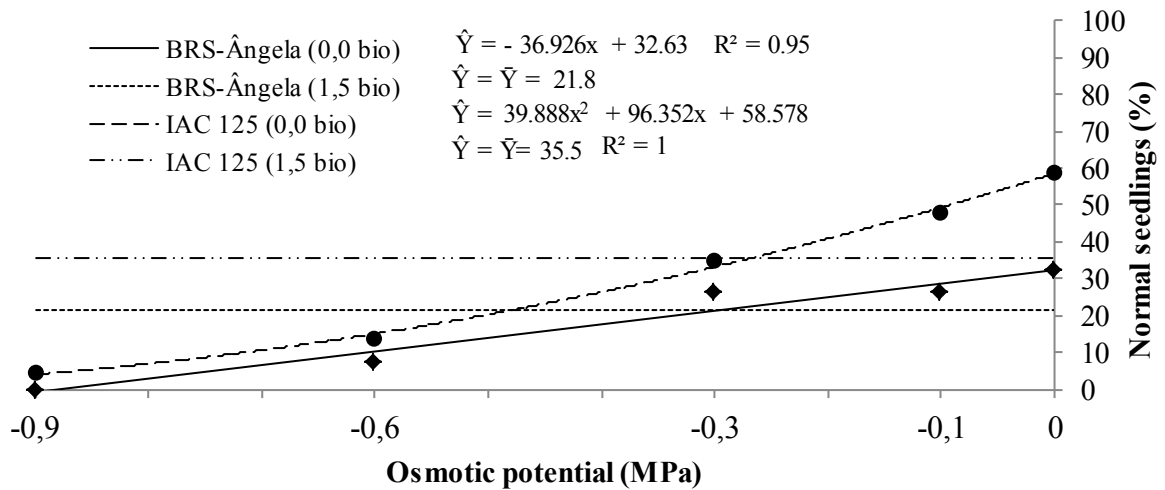
Means followed by the same letter in the column do not differ at 5% probability by the F test.

Table 1 shows the first germination count, in which there was an effect of the bioregulator application only in the control for the two cultivars tested. These results corroborate those observed by Moterle et al. (2011) when testing different doses of this product in soybean seeds. For the authors, the phytohormones present in the growth regulator acted on cell division and growth, promoting vigor increase.

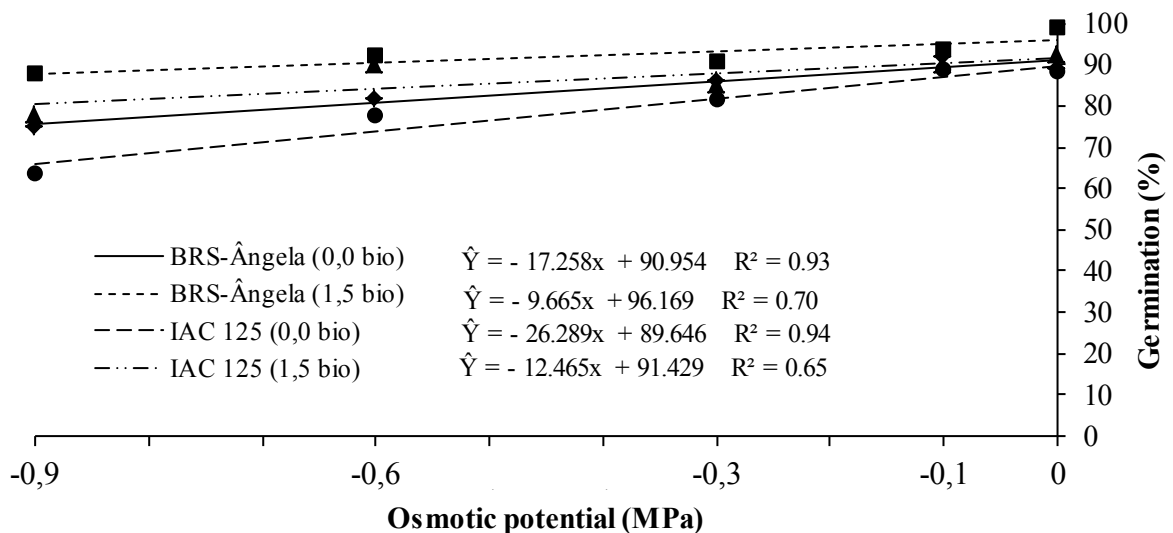
A more marked reduction was observed for cultivar BRS-Angela compared to IAC 125 when seeds were not treated with bioregulator, considering the percentages of normal seedlings in the first count, in face of the decrease in mannitol osmotic potential levels (Figure 1A).

**Figure 1.** Normal seedling percentages in the first (A) and final (B) counts of the seed germination test for two popcorn cultivars submitted to five levels of osmotic potential in a mannitol solution.

A



B



Reductions in the corn seeds vigor obtained by the first count were also found in other studies. Vaz-de-Melo et al. (2012) evaluated simulated water restriction with PEG 6000 solutions and reported a reduction in germination percentage in the three cultivars used. Similarly, Kappes et al. (2010) reported differentiated behavior among corn cultivars under stress conditions. No significant difference was found between the cultivars in the lower osmotic potential levels. However, the seeds of hybrids XB 6010 and XB 9003 presented less vigor under 0.6 MPa. While in the most negative potential (-1.2 MPa), there was a drastic reduction in the percentage of normal seedlings, in which the seeds of XB 6010 and XB 6012 cultivars had lower germination potential.

Regarding germination, although there was a reduction in the percentage of normal seedlings as a result of increased water stress levels (Figure 1B), the bioregulator treatment yielded favorable results in potentials of -0.6 and -0.9 MPa in the two cultivars evaluated (Table 1).

The results in Figure 1B show that, when the seeds were treated with growth regulator, every 1.0 MPa fall in osmotic potential was responsible for a germination decrease of 9.67% (angular coefficient) for the cultivar BRS-Ângela and of 12.47% for IAC 125. Similarly, the use of growth regulator had a positive effect on the germination of onion seeds (LESZCZYNSKI et al., 2012), (SANTOS et al., 2013) and rice (ELLI et al., 2016), but it was indifferent in wheat seeds Abati et al. (2014) and corn (BARBIERI et al., 2014). Due to the results obtained here, it is worth mentioning that the beneficial effects of bioregulator use become more evident when germination conditions are less favorable. In the case of this study, it was proved by an increase in water stress promoted by mannitol.

According to Stenzel et al. (2003) and Schwechheimer (2008), gibberellin is one of the main hormones responsible for germination, since it stimulates the synthesis and activity of hydrolase

enzymes by the cells of the aleurone layer. These enzymes act in the digestion of reserves, that is, in the degradation of starch and proteins into soluble sugars and amino acids, promoting the embryo's growth and development. During this process, auxins and cytokinins also play essential roles as stimulators of membrane permeability and cell division, respectively, promoting seedling germination and development (TAIZ; ZEIGER, 2009).

Under stress conditions, the regulatory substances in the bioregulator, combined with other substances (amino acids, nutrients, and vitamins), may have acted during germination process and in embryo reserve mobilization, growth, and development (VIEIRA; MONTEIRO, 2002).

It is also worth noting that up to the potential -0.9 MPa in cultivar BRS-Ângela, and -0,6 MPa in cultivar IAC 125, the germination percentage with bioregulator application remained above the minimum percentage for commercialization, which is 85% (Table 1 and Figure 1B) (BRASIL, 2009). These results agree with Conus et al. (2009), who observed that corn is moderately tolerant to salt stress in the germination phase. Similarly, Barbieri et al. (2014) observed that up to potential -0.4 MPa, the germination percentage of hybrids was also above the minimum percentage for commercialization. However, with increasing stress, from -0.4 MPa on germination was significantly reduced, thus affecting the germination process speed. High concentrations of salt in plants can be toxic, and in the soil can make root water extraction difficult. Thus, an efficient regulatory system is essential to keep the concentration of toxic ions low and increase the presence of essential ions (MUNNS; TESTER, 2008).

Table 1 also shows that in more negative potentials, -0.6 and -0.9 MPa, the bioregulator allowed significantly higher results in the physiological potential (shoot and root length, and dry biomass). Abati et al. (2014) verified that the

reduction in the solution osmotic potential from -0.4 MPa on affected seedling length compared to the control, and shoot length showed a higher decrease rate compared to that of roots. In addition, it is important to note that the seedling length was more sensitive to reduced water availability compared to germination.

The use of the combination gibberellin ( $GA_3$ ) versus cytokinin (ARAGÃO et al., 2001) and  $GA_3$  versus scarification and maceration (ARAGÃO et al., 2006) provided higher growth of sweet corn and watermelon seedlings, respectively. Gibberellins stimulate the synthesis and activity of enzymes and favor cell expansion by increasing length and cell number (TAIZ; ZEIGER, 2009). Thus, it is likely that these hormones contributed to the higher seedling growth rates.

As emphasized by Vieira and Castro (2004), the bioregulator acts efficiently and effectively on several fundamental physiological processes, such as initial seedling vigor and the production of organic compounds. This may explain the greater length and dry biomass of seedlings under stress conditions. According to Leite et al. (2003), exogenous application of gibberellin in seeds provides a restricted hypocotyl increase, not reflecting on plant height.

These results clearly show that under normal water availability conditions, the bioregulator is inefficient in improving physiological potential. However, under field conditions, where water may be limited, the use of growth regulator improves the initial growth and performance of popcorn seedlings.

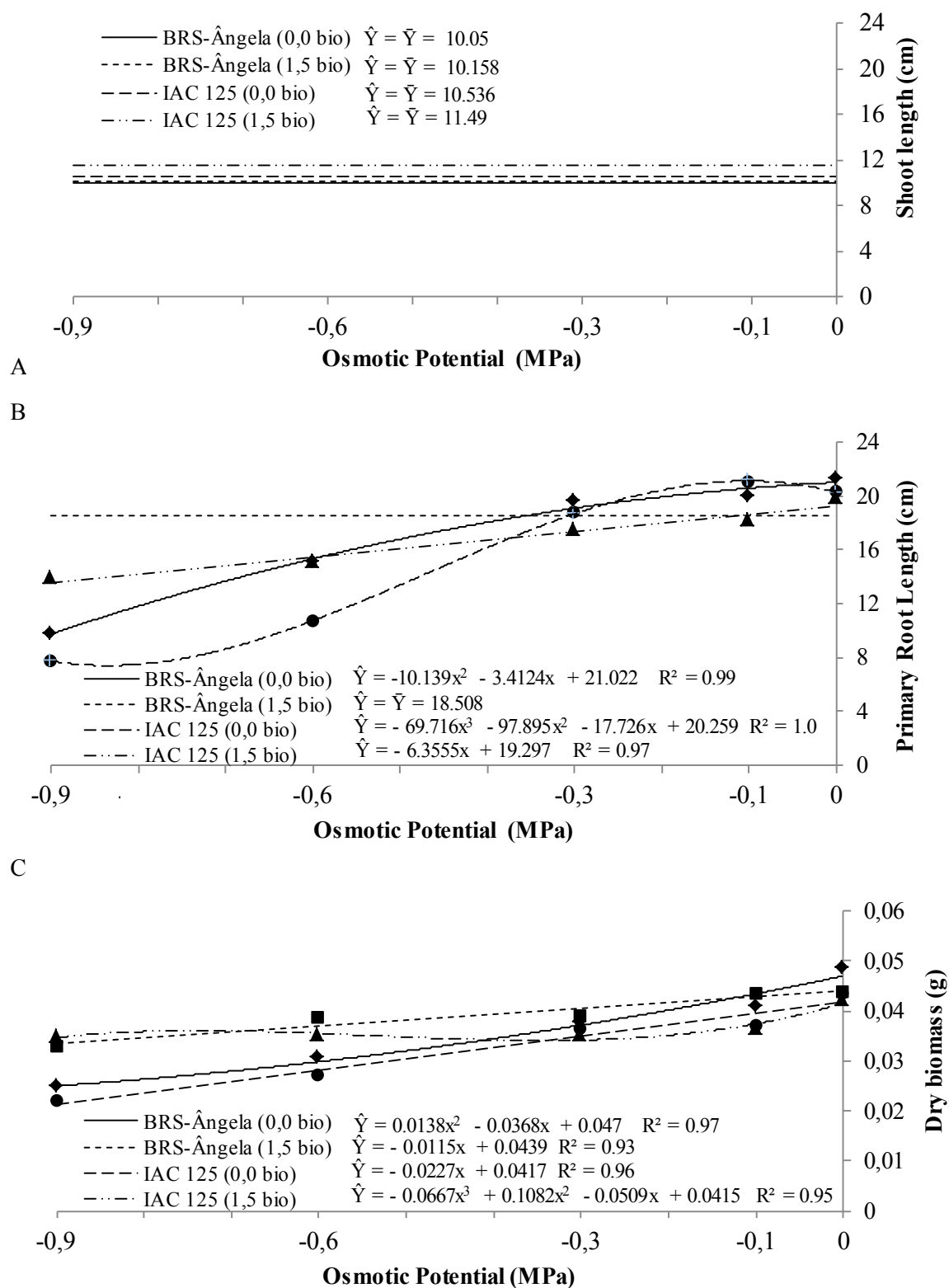
The fact that exogenously applied phytohormones are not completely absorbed by seeds should be emphasized. The seed contact surface, the amount of water, and the solution concentration determine this absorption rate (BUCHANAN et al., 2000).

Figure 2 shows the length results of shoots, primary root, and dry biomass of seedlings of two popcorn cultivars treated with bioregulator and submitted to water stress.

No significant difference was observed in the decrease of osmotic potential levels for the shoot length of popcorn cultivar seedlings. These results contradict those observed in seedlings of the same species (MOTERLE et al., 2006; BARBIERI et al., 2014) and wheat (ABATI et al., 2014), which showed reduced development due to elevated water stress. However, treatments with bioregulator were verified to allow greater seedling shoot growth, especially in more negative potentials (-0.3, -0.6, and -0.9) (Table 1). The hormones present in Stimulate® were probably readily absorbed, improving seedling hormonal balance, thus contributing to the elongation of cells and seedlings.

The length of seedling primary roots was influenced by the increase of osmotic potential levels (Figure 2B). According to Abati et al. (2014), water deficiency conditions restrict seedling growth by slowing down physiological and biochemical processes. Growth reductions are associated with turgidity levels that are compromised in these stress situations (TAIZ; ZEIGER, 2009). Even with a decrease in the mentioned variable as a function of stress, the treatments using bioregulator at the lowest potentials promoted longer roots for both cultivars. These results corroborate those observed by Custódio et al. (2009) and Barbieri et al. (2014), for bean and corn seeds, respectively. According to Toorchi et al. (2009), a well-developed root system is an efficient mechanism in plant resistance to overcome osmotic stress, because under these conditions the adjustments in shoot expansion are due to the amount of water accumulated in the roots, allowing plant development. Thus, the use of the bioregulator as a stimulant of the root system growth may have contributed to greater water stress tolerance.

**Figure 2.** Length of shoots (A) and primary roots (B), and dry biomass (C) of seedlings of two popcorn cultivars submitted to five levels of osmotic potential in a mannitol solution.





Considering dry biomass (Figure 2C), the cubic regression adjustment for cultivar IAC 125 treated with bioregulator evidenced the attempt of seedlings to overcome higher stress levels. On the other hand, cultivar BRS-Ângela showed linear biomass decrease due to the increase of osmotic potential levels. However, even with such a reduction, the positive effects of the growth regulator were confirmed under these conditions. The increase in root length, caused by the application of gibberellin and auxin (VIEIRA; MONTEIRO, 2002), probably reflected in higher biomass accumulation, mainly under high water stress conditions.

The comparison of the cultivars showed great variability. In general, this study showed a greater prominence for the cultivar BRS-Ângela compared to the hybrid IAC 125.

The superiority of the BRS-Ângela cultivar was also observed and highlighted by Moterle et al. (2006). Because it is a genotype with a broader genetic base, it also tends to present greater genetic plasticity in relation to environmental conditions (ALLARD, 1960; PINTO, 2009). While the hybrid with the less genetic constitution, therefore with a narrower genetic basis, does not tolerate higher stress levels.

## Conclusions

The treatment of popcorn seeds with bioregulator is not recommended under normal conditions or with little water restriction, but under conditions of limited water, the use of bioregulator positively influences seedling germination, growth, and initial performance.

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